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Polysaccharide glucomannan isolated from *Heterodermia obscurata* attenuates acute and chronic pain in mice

Marina M. Córdova[a,b], Daniel F. Martins[a,b], Morgana D. Silva[a,b], Cristiane H. Baggio[c], Elaine R. Carbonero[d], Andrea C. Ruthes[d], Marcello Iacomini[d], Adair R.S. Santos[a,b,*]

[a] *Laboratório de Neurobiologia da Dor e Inflamação, Departamento de Ciências Fisiológicas, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil*
[b] *Pós-Graduação em Neurociências, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil*
[c] *Departamento de Farmacologia, Universidade Federal do Paraná, Curitiba, PR, Brazil*
[d] *Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná, Curitiba, PR, Brazil*

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ABSTRACT

Glucomannan (GM) is a polysaccharide obtained from *Heterodermia obscurata* lichens. The present study was conducted to elucidate the antinociceptive effect of GM in behavioural models of acute and chronic pain in mice. GM reduced mechanical allodynia and the levels of interleukin 1-β (IL-1β) in spinal cord and nerve in the partial sciatic nerve ligation (PSNL) model. Systemic treatment with GM inhibited the nociception induced by intraplantar injection of glutamate and by intrathecal injection of *N*-methyl-D-aspartic acid (NMDA), (±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (*trans*-ACPD), tumour necrosis factor α (TNF-α) and IL-1β. Taken together, our data demonstrate that GM has significant antinociceptive effect in acute and chronic pain, suggesting a potential interest in the development of new clinically relevant drugs for the management of pain.

1. Introduction

The sensation of pain alerts us to real or impending injury and triggers an appropriate protective response. Unfortunately, pain often outlives its usefulness as a warning system and instead becomes chronic and debilitating (Julius & Basbaum, 2001). Altered activity of spinal and/or brain neurons relevant to the pain pathway, and also of primary sensory nociceptors, leads to chronic neuropathic pain (Milligan & Watkins, 2009). However, data from clinical trials and experience indicate that treatment of neuropathic pain presents a considerable challenge, because many patients do not experience sufficient pain relief. This may be due to the heterogeneity of neuropathic pain mechanisms, and the frequently coexisting psychological and emotional aspects of chronic pain (Baron, Binder, & Wasner, 2010). For this reason, the development of analgesic drugs requires the testing of new therapeutic sources and with few side effects.

Physicians have long relied on natural products (e.g., chemical compounds produced by animals, plants, and microorganisms) to treat diseases. Natural products are directly or indirectly responsible for approximately 50% of all drugs currently in use (Koehn & Carter, 2005). In this respect, some lichenized fungal species have been used in traditional medicine and it was found considerable quantities of polysaccharides (Baron, Iacomini, Fant, & Gorin, 1991; Vartia, 1973). Many of them are known to exert anti-tumour, antiviral, immunostimulating or memory-enhancing properties among other biological activities, exhibiting low toxicity levels (Olafsdóttir & Ingólfsdóttir, 2001). Also, it has been shown that the polysaccharides have anti-inflammatory and analgesic effects (Baggio et al., 2010; Komura et al., 2010; Smiderle, Olsen, Carbonero, Marcon, et al., 2008; Smiderle, Olsen, Carbonero, Baggio, et al., 2008). Among these polysaccharides, a glucomannan (GM) isolated from the highly branched lichen species *Heterodermia obscurata* presented antinociceptive and anti-inflammatory effects, with dose-dependent inhibition of acetic acid-induced visceral pain, and also reduction of leucocyte migration (Pereira et al., 2010). However, the mechanisms underlying these effects remain unclear.

Abbreviations: CNS, central nervous system; DRG, dorsal root ganglion; GM, glucomannam; IL-1β, interleukin 1-β; NMDA, *N*-methyl-D-aspartic acid; PSNL, partial sciatic nerve ligation; PMSF, phenylmethylsulphonyl fluoride; TNF-α, tumour necrosis factor α; *trans*-ACPD, (±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid.

* Corresponding author at: Departamento de Ciências Fisiológicas, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, 88040-900 SC, Brazil. Tel.: +55 48 3721 9352x206; fax: +55 48 3721 9672.
E-mail addresses: adair.santos@ufsc.br, adairrs.santos@gmail.com (A.R.S. Santos).

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In the present study, we evaluated the antinociceptive role of GM, using behavioural models of acute and chronic pain in mice. We investigated the effects of GM on the mechanical allodynia response induced by PSNL (neuropathic pain model) and the levels of pro-inflammatory cytokines (IL-1β and TNF-α) in the spinal cord and sciatic nerve. To support the possible involvement of the glutamatergic system and pro-inflammatory cytokines in GM-mediated analgesia, we also investigated the effects of GM on the nociceptive response induced by intrathecal injection (i.t.) of ionotropic (NMDA and kainate) and metabotropic (*trans*-ACPD) glutamate receptor agonists, and also of IL-1β and TNF-α.

2. Materials and methods

2.1. Animals

Male Swiss mice (25–35 g) were obtained from the Federal University of Santa Catarina. The mice were maintained at constant room temperature (22 ± 2 °C) under a 12-h light/dark cycle (lights on at 6:00 AM), with access to food and water ad libitum. Mice (6–10 animals per group) were used only once and were acclimatized to the laboratory for at least 1 h before testing. All testing was conducted during the light cycle. The experimental protocol was approved by the Institutional Ethics Committee for Animal Research of the Federal University of Santa Catarina (protocol number 23080.005796/2010-20/UFSC). All experiments were conducted in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals (Zimmermann, 1983). The number of animals and the intensities of noxious stimuli used were the minimum necessary to demonstrate a consistent effect of the treatment.

2.2. Isolation and characterization of polysaccharide

The polysaccharide GM was extracted, isolated and structurally characterized by Pereira and colleagues, exactly as described in a previous work (Pereira et al., 2010). The dried lichen thallus of *H. obscurata* (30.3 g) was first extracted with acetone at 50 °C for 6 h (2×) and then with EtOH at 60 °C for 6 h (2×), in order to extract low molecular components. The defatted product was then submitted to successive aqueous (100 °C) and alkaline extractions (2% and 10% aq. KOH at 100 °C for 6 h, each), and the extracted polysaccharides were recovered by EtOH precipitation (aqueous extract) or after dialysis (HOAc neutralized alkaline extracts), giving fractions W, K2, and K10 respectively. The crude fractions obtained from aqueous (W) and alkaline extractions (K2 and K10) were submitted to freezing followed by gentle thawing at 4 °C (Gorin & Iacomini, 1984), furnishing cold-water soluble (SW, SK2, and SK10) and insoluble polysaccharides, which were separated by centrifugation (8500 rpm, 20 min, 25 °C). The water-soluble fractions were then treated with Fehling solution (Jones & Stoodley, 1965) and the resulting insoluble Cu^{2+} complexes were isolated by centrifugation under the above conditions. The respective complexes (FP-W, FP-K2, and FP-K10), were each neutralized with AcOH, dialyzed against tap H_2O and deionized with mixed ion exchange resins.

The presence of glucomannan (GM) was detected at fraction FP-K2 (Pereira et al., 2010), and the characterization of the structure of GM was based on its monosaccharide composition, methylation, partial acid hydrolysis, and NMR spectroscopic analysis (Pereira et al., 2010). The glucomannan consists of a main chain of (1→6)-linked α-D-mannopyranosyl units, almost all being substituted at *O*-2 with α-D-glucopyranosyl, α-D-mannopyranosyl, and 4-*O*-substituted α-Man*p* groups (Pereira et al., 2010).

2.3. Partial sciatic nerve ligation (PSNL)

Surgical procedures were performed under deep anaesthesia induced by a premixed solution containing ketamine (80 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.), and maintained with isoflurane (2–3% in 100% O_2). Partial ligation of the sciatic nerve was performed by tying the distal 1/3 to 1/2 of the dorsal portion of the sciatic nerve (Malmberg & Basbaum, 1998). In sham group, the sciatic nerve was exposed without ligation. All wounds were closed and covered with iodine solution. Sham-operated mice received only saline (10 mL/kg, i.p.) and the operated mice were randomly divided into control and treatment groups, which received saline (10 mL/kg, i.p.) and GM (100 mg/kg, i.p.), respectively, 7 days after surgery. The mechanical allodynia response was recorded before surgery (B), and immediately before (0 h) and after (0.5 h, 1 h, 2 h, 4 h, and 24 h) treatment to verify the time-course effect of GM. To investigate the effects of long-term treatment with GM on the mechanical allodynia response, GM (100 mg/kg) was administered i.p. once a day for 13 days. The mechanical allodynia response was evaluated 0.5 h after treatment. Repeated treatment was extended from the 7th to the 13th day after ligation, and was then interrupted for 5 days. After this, it was re-initiated to assess the development of possible GM tolerance.

The mechanical allodynia response was evaluated using calibrated von Frey filaments (Stoelting, Chicago, IL), according to the methodology described by Chaplan, Bach, Pogrel, Chung, and Yaksh (1994), with minor modifications. The paw withdrawal threshold was determined using an adaptation of the Dixon up–down method (Chaplan et al., 1994). Mice were further acclimatized in individual clear boxes (9 cm × 7 cm × 11 cm) on an elevated wire-mesh platform, to allow access to the ventral surface of the hind paws. The von Frey filaments (0.02–4.0 g) were presented perpendicularly to the plantar surface of the selected hind paw, and held in this position with enough force to cause a slight bend in the filament. Positive responses included an abrupt withdrawal of the hind paw from the stimulus, or flinching behaviour immediately following removal of the stimulus.

2.4. Determination of pro-inflammatory cytokine levels in the sciatic nerve and spinal cord of the PSNL model

In this series of experiments, we examined the levels of IL-1β and TNF-α *in the sciatic nerve and spinal cord* of the PSNL model of neuropathic pain. Briefly, at the end of the experiment described above, the animals were anaesthetized with isoflurane and killed by decapitation and the sciatic nerve and lumbar portion of the spinal cord (L1–L6) were removed. The tissues were homogenized with a PBS solution containing Tween 20 (0.05%), 0.1 mM phenylmethylsulphonyl fluoride (PMSF), 10 mM EDTA, 2 ng/mL aprotinin, and 0.1 mM benzemethonium chloride and centrifuged at 3000 × *g* for 10 min at 4 °C. The supernatant obtained was stored at (70 °C until further analysis and the total protein content was measured using the Bradford method. The levels of TNF-α and IL-1β were determined using sample aliquots of 100 μL and mouse cytokine ELISA kits from R&D Systems (Minneapolis, MN, USA), according to the manufacturer's instructions.

2.5. Measurement of locomotor activity

To evaluate the possible nonspecific muscle-relaxant or sedative effects of GM, mice were submitted to the rota-rod test, with minor modifications (Scheidt et al., 2002). The apparatus consisted of a bar with a diameter of 2.5 cm, subdivided into 4 compartments by discs 25 cm in diameter. The bar rotated at a constant speed of 17 revolutions/min. The animals were selected 24 h previously by eliminating those mice which did not remain on the bar for 2

consecutive periods of 60 s. Mice were treated by the i.p. route with GM (50 mg/kg and 100 mg/kg) or saline (10 mL/kg) 0.5 h before being tested. The results are expressed as the time (s) for which animals remained on the rota-rod. The cut-off time used was 60 s.

2.6. Glutamate-induced nociception

Glutamate (20 μL; 20 μmol per paw, prepared in isotonic saline solution, with the pH adjusted to 7.4 by the addition of NaOH) was injected intraplantarly into the right hind paw. Animals were observed individually for 15 min after glutamate injection (Meotti et al., 2009). The amount of time spent licking the injected paw was recorded with a chronometer, and was considered to be indicative of nociception. Animals were treated with GM (10–100 mg/kg, i.p.) 0.5 h before glutamate injection. Control animals received a similar volume of saline (10 mL/kg, i.p.).

2.7. Intrathecal injection of excitatory amino acids and pro-inflammatory cytokines to induce pain behaviour in mice

Intrathecal injections were given to fully conscious mice. Briefly, the animals were manually restrained, and a 30-gauge needle connected by a polyethylene tube to a 25 μL Hamilton gas-tight syringe (Hamilton, Birmingham, UK) was inserted through the skin and between the vertebrae into the subdural space of the L5–L6 spinal segments. Intrathecal injections (5 μL/site) were administered over a period of 5 s (Hylden & Wilcox, 1980). Biting behaviour was defined as a single head movement directed at the flanks or hind limbs, resulting in contact of the animal's snout with the target organ. The nociceptive response was elicited by NMDA (450 pmol/site, a selective agonist of the NMDA glutamatergic ionotropic receptor), kainate (110 pmol/site, a selective agonist of the kainate subtype of glutamatergic ionotropic receptors), and *trans*-ACPD (50 nmol/site, a non-selective agonist of metabotropic glutamate receptors, which is active at group I and group II) (Scheidt et al., 2002), TNF-α (0.1 pmol/site) and IL-1β (1 pmol/site) or saline (5 μL/site, i.t.) (Paszcuk et al., 2007). The amount of time the animal spent biting or licking the caudal region was taken as evidence of nociception and was evaluated following local post injections of the following agonists: NMDA (5 min), kainate (4 min), and *trans*-ACPD (Scheidt et al., 2002) TNF-α, and IL-1β (15 min) (Paszcuk et al., 2007). Animals received GM (100 mg/kg, i.p.) 0.5 h before intrathecal injection of 5 μL of the drugs, while control animals received a similar volume of saline (10 mL/kg, i.p.).

2.8. Drugs and reagents

The following substances were employed: L-glutamic acid hydrochloride (glutamate) from Sigma Chemical Co. (St. Louis, USA); *N*-methyl-D-aspartic acid (NMDA), kainite, and (±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (*trans*-ACPD) from Tocris Cookson Inc. (Ellisville, USA); and interleukin 1-β (IL-1β) and tumour necrosis factor α (TNF-α) from R&D Systems (Minneapolis, USA).

2.9. Statistical analysis

The results are presented as mean ± standard error of the mean (SEM), except for the ID_{50} values (e.g., the dose of GM that reduced the nociceptive response by 50% in relation to the control value), which are reported as geometric means accompanied by their respective 95% confidence limits. The statistical difference between groups was analyzed by one-way ANOVA followed by the Newman–Keuls test, or two-way ANOVA followed by the Bonferroni test for the chronic treatment. Differences were considered to be statistically significant at $p < 0.05$. The ID_{50} value was determined by nonlinear regression from individual experiments, using Graph-Pad software (GraphPad Software, San Diego, CA). The percentages of inhibition were calculated for the most effective dose used.

3. Results

3.1. GM decreases the mechanical allodynia response caused by PSNL

In comparison with the sham group, the PSNL model showed reduced paw withdrawal threshold as a measure of the mechanical allodynia response to von Frey filament application (Fig. 1). Acute treatment with GM (100 mg/kg), administered by the i.p. route, produced rapid and significant ($p < 0.001$) inhibition of the mechanical allodynia response induced by PSNL, with 100% reversion at 0.5 h after administration (Fig. 1A). Long-term treatment of animals with GM (100 mg/kg, i.p.) once a day markedly reduced the mechanical allodynia response induced by PSNL, and this effect was evident until the 13th day of treatment. When the treatment was suspended for 5 days, the mechanical allodynia response was re-established, showing that GM treatment did not alter sensory thresholds (Fig. 1B). On the 19th day, treatment with GM was restarted. Once again, the mechanical allodynia response was significantly ($p < 0.001$) reduced, indicating that GM did not lead to the development of anti-allodynia tolerance, nor did it alter sensory thresholds. Analysis of the area under the curve showed that acute and long-term treatment of animals with GM significantly affected the mechanical allodynia response of the PSNL group (Fig. 1C and D). Acute and long-term treatment of sham animals with GM (100 mg/kg) did not alter the sensory threshold when compared to the sham group treated only with saline (data not shown).

3.2. Effect of GM on levels of pro-inflammatory cytokines in the sciatic nerve and spinal cord after PSNL

PSNL model significantly increased ($p < 0.05$) the levels of IL-1β and TNF-α in the sciatic nerve and spinal cord when compared with the sham group (Fig. 2). Long-term treatment with GM (100 mg/kg, i.p.) markedly reduced the levels of IL-1β in the sciatic nerve (99 ± 7%), and also in the spinal cord (63 ± 17%), compared with the PSNL group (Fig. 2C and D). However, the same dose of GM (100 mg/kg, i.p.) reduced (although not significantly) the level of TNF-α in the spinal cord (63 ± 25%; Fig. 2B), but not in the sciatic nerve (Fig. 2A) when compared with the PSNL group.

3.3. GM reduces glutamate-induced nociception

GM (10–100 mg/kg, i.p.), administered 0.5 h before testing, induced a dose-related inhibition of glutamate-induced nociception with ID_{50} value of 34.1 (26.0–44.6) mg/kg and the inhibition of 81 ± 5% for the dose of 100 mg/kg (Fig. 3).

3.4. Effect of GM on intrathecal injection of excitatory amino acids and pro-inflammatory cytokines

Intrathecal injection of NMDA (Fig. 4A), kainate (Fig. 4B), and *trans*-ACPD, TNF-α, or IL-1β (Fig. 4C) caused significant ($p < 0.001$) biting behaviour in mice compared to animals injected intrathecally with saline. In all groups, systemic pre-treatment with GM (100 mg/kg, i.p.) significantly ($p < 0.001$) reduced the biting behaviour compared to mice treated with saline (10 mL/kg, i.p.). The greatest effect of GM was observed on the pro-inflammatory cytokines and NMDA, with the following inhibition percentages: TNF-α (92 ± 7%), IL-1β (91 ± 5%), NMDA (69 ± 1%), and *trans*-ACPD

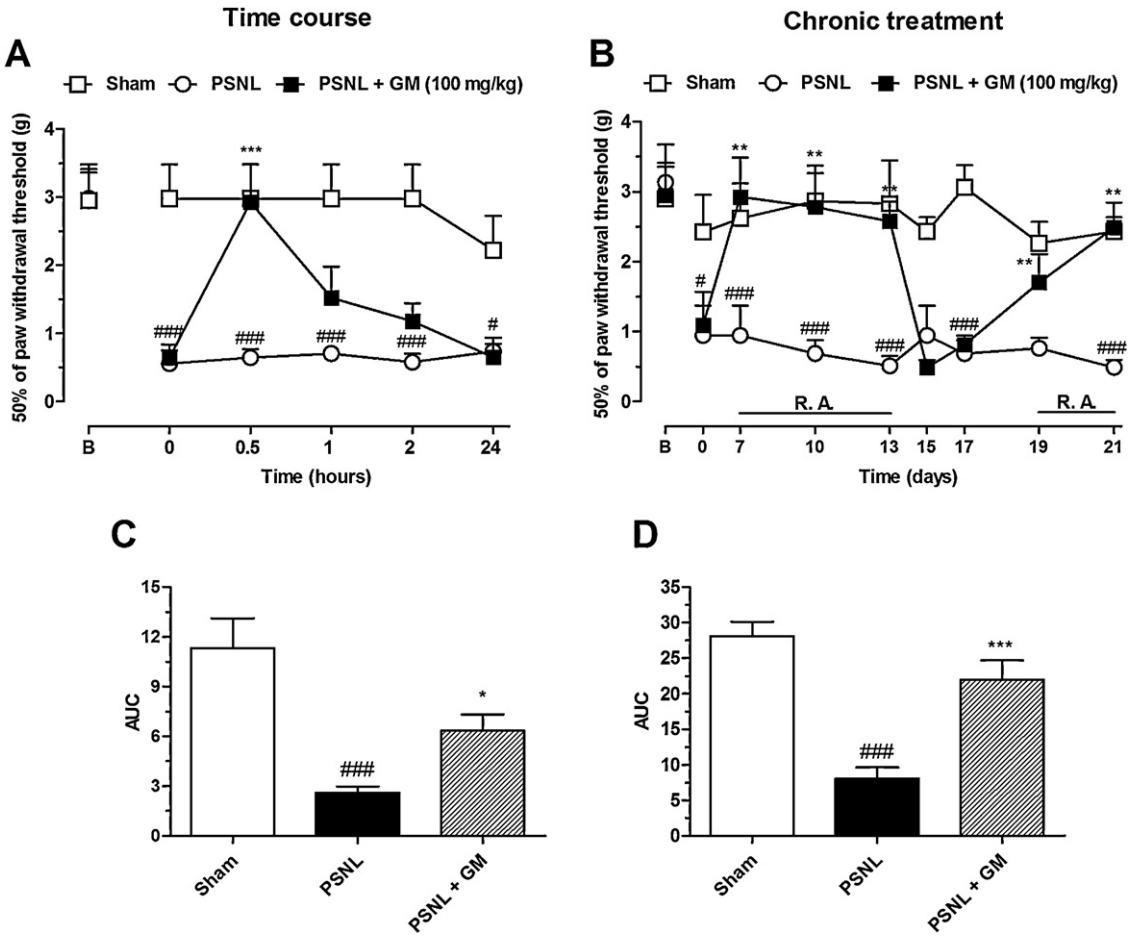

Fig. 1. Effect of acute (panels A and C, analysis of the area under the curve) or chronic administration (panels B and D, analysis of the area under the curve) of GM (100 mg/kg, i.p.) on the mechanical allodynia response induced by partial sciatic nerve injury. Each point represents the mean of 8–10 animals; vertical lines indicate the SEM. The comparison between groups was performed by two-way ANOVA followed by the Bonferroni test. Significance levels in comparison with the PSNL group values are denoted as follows: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. Significance levels in comparison with the sham group values are denoted as follows: $^{\#}p < 0.05$ and $^{\#\#\#}p < 0.001$. RA indicates repeated administration.

(71 ± 12%). By contrast, at the same dose, GM had no significant effect on the kainate-mediated biting response (Fig. 4B).

3.5. Effect of GM on locomotor activity (rota-rod test)

In comparison with saline administration (control group), intraperitoneal administration of GM (50 mg/kg and 100 mg/kg), 0.5 h before testing, did not alter the locomotor activity in the rota-rod test. The residence time for the control group was 60.0 s, while the residence times for the groups treated with GM at doses of 50 mg/kg and 100 mg/kg were 59.7 ± 0.3 s, and 58.8 ± 0.9 s, respectively.

4. Discussion

Polysaccharides isolated from plants and other natural sources exert anti-tumour, immunomodulatory, and anticoagulant activities (Capek et al., 2003; Cipriani et al., 2006; Nergard et al., 2005; Yamada, 1994). Recently, we demonstrated that many fungal-derived polysaccharides exhibit important pharmacological properties, in particular antinociceptive (analgesic) and/or anti-inflammatory activities (Baggio et al., 2010; Carbonero et al., 2008; Smiderle, Olsen, Carbonero, Marcon, et al., 2008; Smiderle, Olsen, Carbonero, Baggio, et al., 2008). Interestingly, we also showed that the polysaccharide glucomannan (GM) isolated from lichenized fungus *H. obscurata* reduced the abdominal contractions induced by acetic acid, with concomitant reduction of leucocyte migration to the peritoneal cavity, indicating that it may have antinociceptive and anti-inflammatory properties (Pereira et al., 2010). For this reason, this study aimed to extend the previous data evaluating the GM effects on acute and chronic pain in mice.

The inhibitory effects of GM on acetic acid-induced nociception could be attributed, at least in part, to a reduction of inflammatory mediator's release. These endogenous inflammatory mediators, such as bradykinin, glutamate, prostanoids, and cytokines (IL-1β and TNF-α), are also released during peripheral nerve injury contributing to the instigation of neuropathic pain (Ji & Strichartz, 2004). To investigate the potential and additional analgesic effects of GM, we used a preclinical neuropathic pain model. We observed that acute or long-term administration of GM significantly reduced the mechanical allodynia response induced by PSNL in mice. This effect was evident throughout the treatment regime but, with suspension of treatment for 5 days, hypersensitivity returned to control levels. However, when treatment was restarted, we observed that GM again reduced the mechanical allodynia response, indicating that GM does not have a cumulative effect. Moreover, it does not lead to the development of anti-allodynia tolerance, nor does it alter sensory thresholds.

Additionally, GM also decreases the levels of the pro-inflammatory cytokine IL-1β in the sciatic nerve and spinal cord after PSNL. However, treatment with GM also inhibited, not significantly, the levels of the pro-inflammatory cytokine TNF-α in

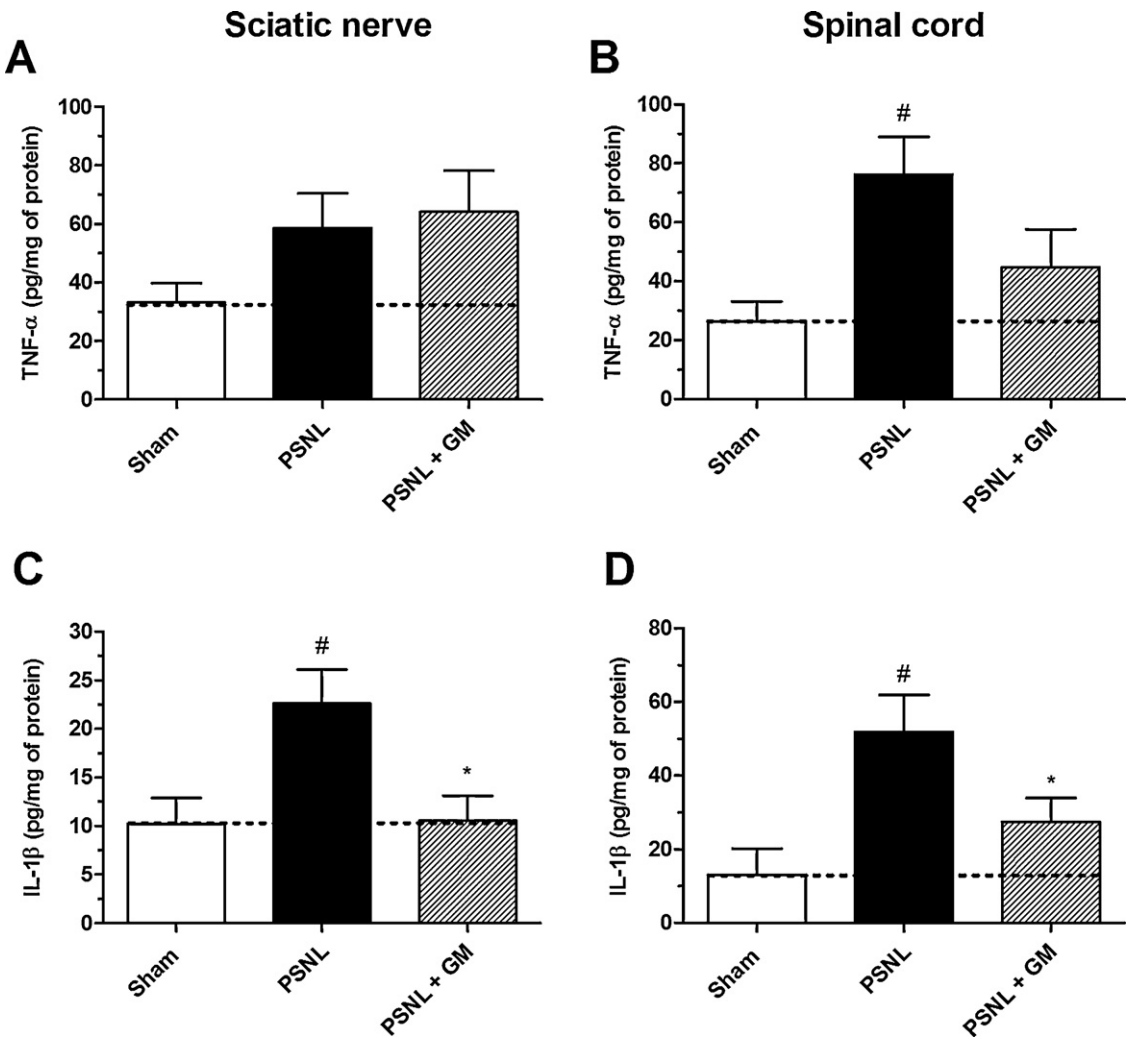

Fig. 2. Effect of GM (100 mg/kg, i.p.) on the levels of pro-inflammatory cytokines induced by partial sciatic nerve injury. Levels of TNF-α and IL-1β in the left sciatic nerve (panels A and C) and the spinal cord (panels B and D). Each column represents the mean of 8 animals; vertical lines indicate the SEM. The comparison between groups was performed by one-way ANOVA followed by the Newman–Keuls test. Significance levels in comparison with the PSNL group values are denoted by $^{*}p < 0.05$. Significance levels in comparison with the line group values are denoted as follows: $^{\#}p < 0.05$.

the spinal cord after PSNL. Following injury, activated microglia and astrocytes in the CNS release large quantities of endogenous mediators (e.g., glutamate and cytokines), which contribute to hypersensitivity and the maintenance of neuropathic pain (Austin & Moalem-Taylor, 2010). Interestingly, systemic treatment with GM also markedly reduced the nociceptive behaviour induced by intrathecal injection of IL-1β and TNF-α, confirming the involvement of pro-inflammatory cytokines in GM antinociception.

The ability of pro-inflammatory cytokines (e.g., TNF-α and IL-1β) to induce acute nociception when administered intrathecally has been attributed to the release of glutamate and substance P (SP) from nociceptive terminals (Austin & Moalem-Taylor, 2010). Besides, IL-1β has been shown to increase the currents of ionotropic AMPA and NMDA receptors in the spinal cord, by increasing the influx of Ca^{2+} and activating protein kinase C (PKC), which phosphorylates the subunits NR1 and NR2B of NMDA receptors (Brenner, Ji, Shaffer, & Woolf, 2004; Guo et al., 2002; Kawasaki, Zhang, Cheng, & Ji, 2008). In the present study, we observed that i.p. administration of GM inhibited significantly and in a dose-dependent manner the acute nociceptive behaviour caused by i.p. glutamate injection. This result suggests the involvement of the glutamatergic system in the GM mechanism of action. To evaluate which glutamate receptors (ionotropic or metabotropic) participate in

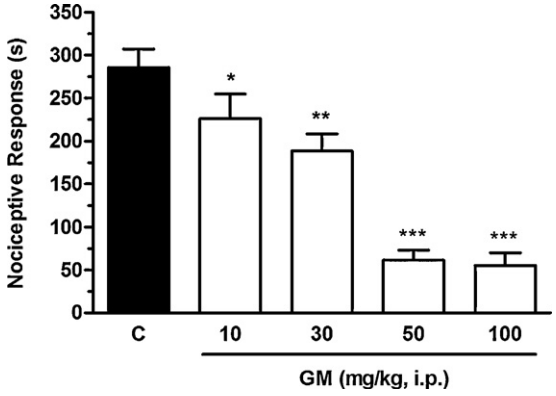

Fig. 3. Effect of GM (10–100 mg/kg, i.p.) on the nociception induced by i.p. injection of glutamate. Each column represents the mean of 6–8 animals; vertical lines indicate the SEM. The comparison between groups was performed by one-way ANOVA followed by the Newman–Keuls test. Significance levels in comparison with the control group values (closed columns) are denoted by: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

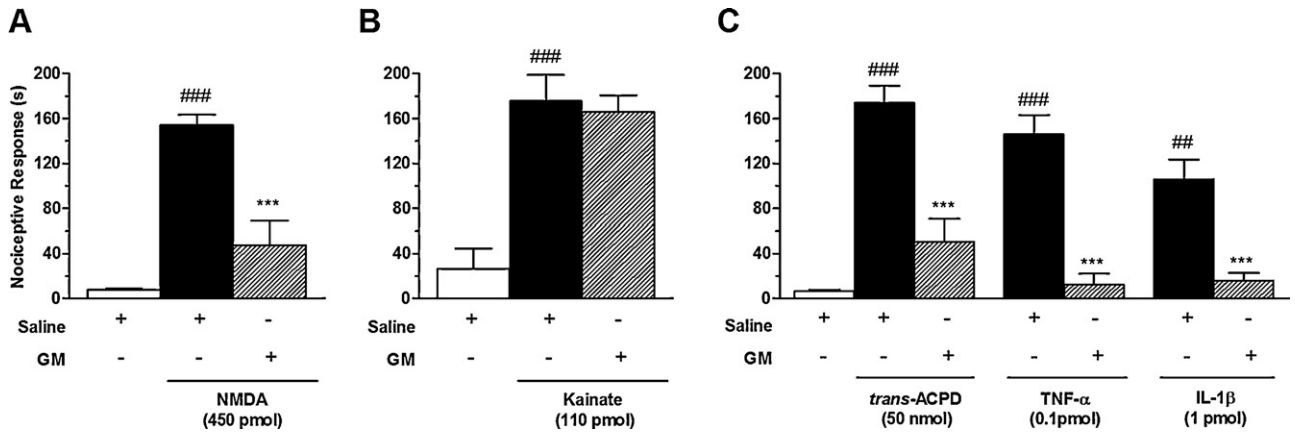

Fig. 4. Effect of GM (100 mg/kg, i.p.) on the biting response caused by i.t. injection of NMDA (panel A), kainate (panel B), and *trans*-ACPD, TNF-α, or IL-1β (panel C). Each column represents the mean of 6–8 animals; vertical lines indicate the SEM. The comparison between groups was performed by one-way ANOVA followed by the Newman–Keuls test. Significance levels in comparison with the control group values (closed columns) are denoted by $^{***}p < 0.001$. Significance levels in comparison with the saline group values are denoted as follows: $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$.

the GM-induced antinociception, we performed the intratecal injection of kainate- and NMDA-receptor agonists. Indeed, the application of NMDA-receptor antagonists has been shown to prevent the development of persistent pain (Suzuki, 2009), and also the central and peripheral sensitization induced by tissue injury or inflammation (Dickenson, Chapman, & Green, 1997; Schaible, Grubb, Neugebauer, & Oppmann, 1991). Our data demonstrated that i.p. treatment with GM inhibited the nociception induced by NMDA receptor agonist, but did not alter the response evoked by kainate, indicating the regulation of NMDA receptor pathway by GM. Differently from a β-glucan isolated from the mushroom *Pleurotus pulmonarius* that only inhibited the ionotropic glutamate receptor-induced nociception (Baggio et al., 2010), GM also reduced the nociception induced by *trans*-ACPD (a group I and group II metabotropic-receptor agonist). However, given that there is an interaction between ionotropic (NMDA) and metabotropic receptors in the nociceptive process, additional studies, such as specific binding and administration of specific antagonists, are required to confirm the interaction of these glutamate receptors with GM.

Another interesting point is that GM did not cause motor deficit, nor did it change the locomotor performance of mice in the rota-rod test. This suggests that GM antinociception is not associated with nonspecific effects. In this respect, it is important to note that various substances (e.g., ketamine, memantine, and amantadine) that antagonize NMDA receptors (Parsons, 2001) or mGluR I (Pietraszek, Nagel, Gravius, Schäfer, & Danysz, 2007; Takahashi & Afford, 2002) produce significant changes in locomotor activity. Our results indicate that GM treatment produces antinociceptive actions in specific models of nociception induced by glutamate, NMDA, and *trans*-ACPD, without harming locomotion.

5. Conclusion

In summary, our present data confirm and extend previous results by demonstrating the significant antinociceptive effects of GM in acute and chronic models of nociception. The antinociceptive effects promoted by GM seems to involve the cytokine and glutamate signalling pathways, however, the exactly mechanism of action remains unclear and further studies are being carried out. These findings suggest that GM may constitute an interesting molecule for the development of a therapeutically useful drug to control acute and chronic pain.

Competing interest

The authors declare that no competing interest exists.

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